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Toxic protein spread in neurodegeneration: reality vs fantasy

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Abstract

Over the past decade, the importance of the propagation of amyloidogenic proteins such as α-synuclein and tau in the pathogenesis of neurodegenerative diseases has been supported by numerous neuropathological and experimental studies. While these proteins behave similarly to prions, recent evidence suggests the existence of fundamental differences as they can propagate in the absence of endogenous template, they do not exhibit a strict “strain” behavior, and they may not be transmissible between individuals. We therefore propose to name these proteins “prionoids”. In this review we critically assess the extent of the overlap between these two entities and evaluate how the propagation of prionoids can fit into the wider system dysfunction seen in the brains of patients with Alzheimer’s and Parkinson’s disease.

Keywords: prionoids, neurodegeneration, prions, propagation, Alzheimer’s, Parkinson’s

Prions and prionoids

Braak and colleagues observed that in several neurodegenerative diseases, pathological aggregates of proteins such as α-synuclein and tau colonize the brain following stereotypical patterns and suggested that this phenomenon is a result of a causative “agent” spreading from a starting point throughout the brain [1-3]. It is thought that this agent is misfolded tau and α-synuclein and that the spread of pathology underlies disease progression and concomitant aggravation of the clinical features, a notion that has been termed **“Braak hypothesis” (see Glossary)**. This suggested that those proteins have the ability to transfer from cell to cell [4] , possibly through templated nucleation as previously described for prions [5]. We have therefore designated α-synuclein and tau as “prionoids” [6, 7], since they share **propagative** mechanisms with prions although they may not be transmissible between hosts.

Recent observations have also challenged the Braak hypothesis, and have pointed to radical differences between prions and prionoids [8]. The extent of the functional and biological overlap between the two entities is currently a matter of intensive research and it is anticipated that the conclusions will shed light on the fundamental pathogenetic mechanisms of neurodegenerative diseases. In this article, we discuss whether α-synuclein and tau fulfill the criteria to be designated as bona fide prions, and review recently identified mechanisms that are involved in the initiation and development of tau and α-synuclein pathologies.

Do prionoids possess critical features of bona fide prions?

By definition, a **prion** is a “proteinaceous infectious particle” [9]. This is much more than an academic game of words: prions have been responsible for large, tragic epidemics, such as Kuru and iatrogenic Creutzfeldt-Jakob disease, and for enormous epizootics such as scrapie and bovine spongiform encephalopathy. As long as α-

synuclein, Aβ, tau, and any other aggregating proteins are not shown to be "infectious" in the microbiological sense of the word, it is semantically inappropriate to label them as prions – hence we coined the term "prionoid".

But even if one abstracts from the issue of microbiological infectiousness, there are several additional critical differences which raise questions on the similarity between prions and all other prionoids. We first review which of those critical features of prions are shared by prionoids, in other words whether they can transfer as distinct strains, require endogenous template for transfer, spread from a starting point within the brain or the periphery, or can be transmitted iatrogenically. We then review mechanistic aspects underlying the transfer of prionoids between cells such as the mechanisms of release and uptake and the nature of the transferred species. Finally, we analyze the insight on the biology of propagation gained from genetics and neuropathology studies.

In an attempt to clarify the nomenclature used to describe the various types of movement of prions and prionoids between cells, we have adopted the following terms in this manuscript: We use the term "propagation" only when there is cell-to-cell transfer of a misfolded protein with associated conformational changes of the endogenously expressed template. However, when the protein (misfolded or not) simply moves between cells without permissive templating, or when it is unknown whether permissive templating occurs, we refer to this procedure as "**cell-to-cell transfer**" or simply "transfer". Finally, we use the term "**spread**" to refer to the development of misfolded protein pathology sequentially between neighboring brain regions. As "**prionoid**" we define the ordered protein aggregates that replicate through templated nucleation but are not (or at least not yet shown to be) infectious.

Evidence that prionoids propagate as distinct strains is not conclusive

A defining feature of prions is their ability to propagate as distinct **strains** [10]. The meaning of the term strain, however, has been relaxed over time and no longer reflects what the original concept was meant to convey. A strain is a prion isolate that has specific phenotypic characteristics which, crucially, are maintained upon repeated passages through isogenic hosts (Figure 1) (for a detailed discussion on prion strains we refer the reader to previously published reviews on the topic [11, 12]).

There has been sporadic evidence suggesting that α-synuclein and tau might have strain-like properties. Specifically, it has been shown that intracerebral injection of brain homogenates from human brains with a variety of **tauopathies** has the ability to induce distinct tauopathies in mouse models [13]. Similar findings have been reported for α-synuclein [14-16]. Specifically, it has been shown that synthetic α-synuclein can form a variety of strains that differ in their ability to cross-seed tau in vivo and in vitro and exhibit different profiles following proteinase K digestion [16]. There is limited evidence suggesting that Aβ also forms strains [17, 18], but a thorough characterization of the identified strains is lacking.

A limitation of the aforementioned studies was that they did not show whether the identified “strains” have the ability to sustain their unique properties during several passages over long periods of time in vivo [19], which is a critical property of prion strains. This issue was partially addressed by Sanders et al [20]. The researchers generated cell lines stably overexpressing the aggregation-competent section of tau tagged with YFP and exposed them to tau fibrils through lipofection. This experiment led to the formation of intracellular aggregates that persisted over at least 50 days with passaging of the cells every two days. Single cell analyses showed 20 clones each with unique tau aggregate patterns. Cell lysates from those clones had the

ability to induce the same pattern of pathology upon treatment of naïve cultures and distinct pathologies after intracerebral injections in transgenic tau P301S mice. Those strains were also consistently passaged over several generations in vivo and back in vitro [20]. Even though the aforementioned studies are thorough and their results are interesting, they constitute only preliminary evidence that prionoid strains exist. A more thorough characterization of those strains is needed, along with independent replication of the results by several groups, which is a milestone that has not been achieved yet.

Presence of endogenous template is not necessary for cell-to-cell transfer of prionoids

Prions are unable to transfer from cell to cell in the absence of **endogenous template** [21, 22]. Recent evidence suggests that tau protein does not have this critical feature. Wegmann et al [23] crossed the previously published ECrTgTau mice expressing human tau exclusively in the entorhinal cortex [24] with mice with a tau-null background. Surprisingly, tau could transfer from cell to cell in the absence of endogenous template as efficiently as in the presence of tau in the original ECrTgTau mice, and induced tau pathology that was indistinguishable between the two models [23]. However, in the knock out model, the transfer of tau was not associated with toxicity, indicating that the transmitted protein requires endogenous template to actually acquire pathogenic properties. These observations distinguish two possible states of transferring tau: one pathological, which is related to transfer with associated permissive templating (i.e. propagation), and one physiological that is related just to the transfer of the protein without prion-like properties and functional consequences. Interestingly, however, evidence for transfer of pathogenic tau protein without presence of endogenous template has been found in humans with Pick's

disease whose hallmark neuropathological feature are tufted astrocytes [25]. It is known that astrocytes do not express endogenously tau, therefore the occurrence of tau pathology within those cells is most likely caused by transfer of misfolded protein from neurons, without associated templated nucleation. Therefore, the interpretation of the results reported by Wegmann et al is more complicated and additional experiments would be required to clarify the situation.

To confirm those results, the researchers then designed agenetic reporter capable of identifying donor and recipient cells of tau during the transfer process [23, 26]. This reporter consisted of a GFP fluorophore fused to the tau protein, yet separated by the T2A self-cleaving peptide. The researchers then inserted this reporter into an adeno-associated virus (AAV) and injected it into the entorhinal cortex of wild type (WT) mice expressing endogenous tau or of tau null mice. Staining with a human-specific anti-tau antibody allowed for differentiating cells that expressed tau through transfection (which were double positive for GFP and human tau) from cells that received tau through transfer (which contained human tau but not GFP) (Figure 2). This experiment yielded results similar to those seen in transgenic mice and thus supported the hypothesis that tau is probably distinct from true prions.

Development of α-synuclein pathology in midbrain grafts transplanted in patients with PD: relation to α-synuclein transfer

The original reports of the development of Lewy-body (LB) pathology in neurons grafted into the midbrain of patients with PD were considered by several scientists as the first evidence that α-synuclein aggregates could transfer between brain areas like prions [27, 28]. However, several observations contradict the hypothesis that α-synuclein pathology in midbrain grafts transplanted in patients with PD originates

from the transfer of native, misfolded α-synuclein. First, LBs are concentrated in regions with significant **reactive gliosis** [29, 30]. In one case, LBs congregated within the part of the graft closer to the amygdala, which is a region known to be particularly susceptible to the development of neurodegenerative disease pathology [29]. Second, several patients who received midbrain grafts did not develop LB pathology at almost 20 years after the transplantation [31]. Interestingly, the grafts that were spared LB pathology were those that used dissociated neurons [31, 32], whereas the ones that eventually developed LBs had utilized cellular aggregates or tissue pieces [27, 28, 33, 34]. Third, even within the grafts that are positive for LBs, development of pathology was a rather rare occurrence and neurons generally remained alive, healthy and functional for decades after transplantation [27-29, 31, 32, 34]. These observations argue that the development of LB pathology cannot be attributed (exclusively) to transfer of misfolded α-synuclein from the patients' diseased tissue. Rather, development of LB pathology is most likely a reactive process caused by inflammation related to the grafting procedure or the neurodegenerative process ongoing in the host brain, or it could be related to local microenvironment conditions [29, 33].

The conclusions drawn from the studies on grafted material in the brains of patients with PD can also aid in the interpretation of the results from mouse models. Numerous studies have shown that intracerebral injection of fibrillar α-synuclein in transgenic and non-transgenic mouse mice results in the development of widespread α-synuclein pathology in their brains within weeks to months [35-37]. However, the results from the aforementioned studies on human grafts indicate that this procedure can take decades in humans. This suggests that mouse models might not be physiologically relevant and provide only marginal support for α-synuclein propagation in humans because of the differences in timescale.

No strong evidence supporting iatrogenic transmission of AD and PD

It is well established that Creutzfeld-Jakob disease (CJD) can be transmitted through human cadaveric pituitary growth hormone (c-hGH) administration [38, 39], transplantation of dura matter [40], and contaminated cortical electrodes [41]. To assess whether tau, α-synuclein and Aβ could also be transmitted between individuals, one group [42] studied a series of 6,190 patients who had received c-hGH treatment and looked for evidence of neurodegenerative disease on their death certificates. In addition, they analyzed a series of hypophyseal neuropathological specimens to determine the frequency of neurodegenerative disease-associated pathology in a cohort presumably representative of the c-hGH donor population. This analysis showed that, while pathological species of tau, α-synuclein and Aβ are found within the hypophysis of individuals in the general population and could constitute a source of prionoid transmission during c-hGH administration, there is no evidence of development of neurodegenerative disease in c-hGH recipients. However, this study had several limitations [43]. First, given the long incubation time of neurodegenerative diseases, the subjects might have not been studied long enough after the treatment to allow the development of the disease. Second, no neuropathological assessment of the c-hGH recipients was undertaken, therefore it is not possible to know if the patients were truly free of tau, α-synuclein and Aβ pathology or if they were at the preclinical stage of the disease [43].

Recently, two case series of patients with iatrogenic CJD (iCJD) plus Aβ pathology were reported [44, 45]. Those individuals had either undergone dural grafting [44] or treatment with c-hGH [45-47]. In the dural grafting iCJD group, five of seven patients developed Aβ pathology in the form of cerebral amyloid angiopathy (CAA) within the meningeal vessels and plaques within the gray matter, whereas 4 of 8 iCJD patients

treated with c-hGH developed Aβ pathology typical of AD and CAA [45]. The ages of the patients in both series were between 28 and 63 years old, which is too young for the development of such severe degree of Aβ pathology under conventional situations [45, 48]. An increased frequency of severe Aβ pathology was also seen in young patients who underwent neurosurgical procedures without developing iCJD [49]. The authors argue that these observations are consistent with iatrogenic transmission of Aβ pathology. However, an alternative explanation is that the Aβ pathology developed as a reactive process to the neurosurgical intervention or to intricacies associated with the spread of the misfolded protein from the periphery to the brain during c-hGH administration [44, 45].

Finally, epidemiological studies have found no evidence supporting an infectious nature for α-synuclein, as conjugal transmission of PD has not been observed [50, 51] and the incidence of PD remains relatively stable over time in various ethnical populations [52].

In conclusion, the absence of evidence for infectivity supports the notion that there is a clear distinction between prions and prionoids. However, given that prionoids exhibit some behaviors that are similar to bona fide prions, these agents should still be treated with caution and their potential infectivity should continue being a subject of investigation.

Does pathological α-synuclein spread from the periphery to the brain?

The spread of bona fide prions from extraneural sites to the brain is amply documented [53] and its molecular underpinnings are well understood [54-56]. Therefore, it would be reasonable to hypothesize that a similar phenomenon occurs in PD and AD. According to the Braak hypothesis, α-synuclein pathology starts from

the brain stem within the glossopharyngeal and vagal nerve nuclei or in the olfactory bulb and eventually spreads to the neocortex through the midbrain [1]. Intriguingly, recent studies have identified α-synuclein pathology in the colon and the enteric nervous system in patients with PD [57-65], suggesting that the event triggering the initiation of the disease could occur in the periphery and ascend towards the brainstem through as yet undetermined paths. Consistent with a spread of α-synuclein pathology from the periphery, one study showed that mice injected intramuscularly with α-synuclein fibrils rapidly developed brain pathology [66]. In accordance with this finding, intragastric infusion of **rotenone** in mice was able to induce α-synuclein pathology in the brain [67]. In addition, **vagotomy** may reduce the risk for development of PD in humans [68], arguably because it disrupts the migration pathway of pathological α-synuclein seeds from the periphery to the brain. However, those results did not hold up when following the same cohort over a longer period of time [69]. Hence, no clarity is yet attained on the possible pathways of centripetal α-synuclein spread.

Mechanistic aspects underlying the transfer of prionoids

How is transferred α-synuclein released and taken up by the cell?

Previous studies have indicated that transferred α-synuclein can be released from the cells through various mechanisms that could depend on the aggregation status of the protein [70], such as exosomes [71] and non-canonical vesicle-mediated exocytosis [72], or can be transported between neighboring cells through nanotunnels [73].

Recently, a novel, unconventional mechanism underlying the cellular secretion of α-synuclein mediated by the ubiquitin-specific protease 19 (USP19) was identified.

USP19 helps transport the targeted proteins to the ER. Following deubiquitination, the proteins are secreted from the cells via late endosomes. Interestingly, this pathway is used only for the secretion of α-synuclein and other misfolded proteins but not tau and has been termed misfolding-associated protein secretion (MAPS) [74].

The mechanisms with which propagating α-synuclein is taken up by the recipient cells are still a subject of intensive investigations. However, there is evidence suggesting that those include endocytosis [75], heparan sulfate proteoglycans [76] and various cell surface receptors such as LAG3 [77].

What is the transferred species of tau?

The identification of the species of tau that is transferred between cells has been a matter of intense investigations. Takeda et al [78] used a microfluidics device system along with a HEK FRET biosensor tau **seeding** assay [76, 79] to study the transfer and seeding potential of various tau species derived from cortical extracts from AD and healthy human brains, but also from brain extracts and interstitial fluid from tau-transgenic mice overexpressing P301L (Tg4510) or wild type tau (Tg21221). They found that the same seed- and transfer-competent tau species in all those cases was phosphorylated, high molecular weight (HMW) tau, even though it was present in low abundance. Using the same study systems, this group [80] further showed that a HMW tau species with seeding and transfer potential was also found within the cerebrospinal fluid (CSF) of patients with AD.

These findings were partially consistent with those reported by Mirbaha et al [81] with recombinant tau repeat domain (RD) and tau isolated from human AD brains. Following characterization of its structure and assembly size with a variety of methods including size exclusion chromatography, western blotting and mass

spectrometry, they showed that the minimal unit internalized by HEK cells and primary neurons were tau trimers. The discrepancy between the findings reported by Takeda and colleagues and Mirbaha and colleagues could be attributed to the differences in study systems and sources of tau preparations used.

Finally, a third group reported that monomeric but also aggregated tau can be internalized by iPSc-derived neurons. This group [82] used recombinant monomeric and aggregated tau preparations that were first labelled with a fluorescent dye and added them to the conditioned medium of iPSc-derived neurons. They found that monomeric tau entered the cells in two phases: a rapid endocytosis- and a slower micropinocytosis-like phase. On the other hand, aggregated tau entered the cells using a mechanism consistent with endocytosis. The findings reported by this group [82] contradict those by Takeda et al and Mirbaha et al; however, the discrepancy could be explained by the fact that they conducted their experiments on a different cell type using tau that was tagged with a synthetic fluorophore.

In conclusion, there is still confusion regarding the transferring species of tau. Discrepancies between publications could be attributed to the use of different study systems which, in any case, are artificial systems that probably do not accurately represent the physiology in the human brain. Therefore, extrapolation of the results to humans should be done with caution. In addition, as previously argued, it is possible that the cell-to-cell transfer of prionoids could either be a physiological, benign phenomenon, or a more sinister phenomenon involving permissive templating. without the two situations being mutually exclusive; this could potentially be a factor cofounding the design of experiments and interpretation of their results.

Novel mechanisms mediating the aggregation of tau

Recently, liquid-liquid phase separation (LLPS) was recognized as an important mechanism underlying the initiation of the aggregation for various amyloidogenic proteins within the cell such as FUS [83], hnRNPA1 and TDP43 [84]. The same phenomenon may also be involved in the initiation of tau aggregation, which could represent the first step towards tau cell-to-cell transfer. To test this hypothesis, Wegmann et al used r ecombinant phosphorylated or mutant tau and showed that it can form liquid droplets both in vitro and within cells [85]. HMW tau isolated from AD brains also showed similar properties in vitro. In addition, immunostaining of paraffin embedded brain slices from a patient with AD revealed the presence of droplet-like formations within neurons located in the cortex and hippocampus, consistent with the occurrence of LLPS in vivo. It would be interesting to assess whether disruption of LLPS could halt the aggregation and transfer of tau, and whether α-synuclein also undergoes LLPS during aggregation.

Relation between amyloid aggregation and tau cell-to-cell transfer

The amyloid cascade hypothesis posits that Aβ deposition in the form of plaques precedes and promotes **neurofibrillary tangle** (NFT) formation, therefore initiating the pathogenetic cascade that leads to the development of AD [86]. However, recent observations have brought this hypothesis into question. Here we discuss the evidence for and against the amyloid cascade hypothesis and mechanisms with which Aβ could influence tau transfer.

Recently, two neuropathological diseases overlapping with AD were recognized as separate entities: Primary age-related tauopathy (PART) and pathological ageing. PART is characterized by tau pathology fitting the Braak staging and ranging from mild to severe stage IV, with no or mild Aβ pathology (Thal staging 0 to II) [87, 88].

Affected individuals usually have mild dementia and the clinical features correlate well with the severity of the tau pathology and the degree of hippocampal atrophy [89]. Conversely, in pathological ageing there is significant Aβ pathology but minimal tau pathology in critical regions such as the cortex and limbic system. The individuals with this condition do not have the neuronal loss, dendritic pathology and synaptic abnormalities seen in AD and do not exhibit cognitive decline [90]. Finally, it has been observed that in AD the disease severity correlates well with the severity of tau pathology but not Aβ pathology [91]. These three observations suggest that Aβ deposition is a secondary event with minimal importance in disease pathogenesis, with tau deposition being the main event promoting neurodegeneration and disease progression.

However, there is a plethora of evidence pointing in the opposite direction. First, mutations in genes involved in Aβ metabolism are fully penetrant and lead to early onset AD (EOAD), whereas only risk haplotypes and risk factors within *MAPT* can be found in AD [92, 93]; therefore, the genetic contribution of Aβ seems stronger than that of tau dyshomeostasis. Second, data that is almost 20 years old suggests that Aβ can enhance the formation of tau pathology [94, 95]. This observation was recently revisited and expanded upon by Pooler and colleagues, aiming to assess the effect of Aβ not just on tau pathology but also on tau transfer [96]. The researchers crossed rTgTauEC mice [24] with APP/PS1 mice developing Aβ pathology at a young age. At 16 months of age, the mice showed a significant increase in the number of cells within the dentate gyrus and CA1 region of the hippocampus that were positive for tau when compared to rTgTauEC mice, suggesting an increase in cell-to-cell transfer [96].

Researchers from the same group [97] further assessed this phenomenon by using a previously developed tau HEK biosensor FRET system [76, 79]. Brain homogenates

from human brain samples with tau pathology with and without coexistence of Aβ pathology were subjected to the tau seeding assay which showed that tau from human brain samples with Aβ plaques could induce seeding more efficiently compared to tau from cases without Aβ plaques. The same effect was also seen when using brain lysates from APP/PS1xrTg4510 mice exhibiting both plaques and tangle pathology vs brain lysates from rTg4510 mice with only tangle pathology [97]. The enhanced seeding effect was in fact seen even before the mice developed plaques.

More recently, researchers from a different group provided extensive in vivo evidence supporting the observations detailed above [98]. He et al. injected wild type mice and APP-KI mice (which develop Aβ plaques but do not overexpress tau) with sarkosyl-insoluble tau extracted from human AD cases. The APP-KI mice developed tau pathology in the vicinity of Aβ plaques. They also found that the microenvironment around the Aβ plaques was exceedingly conducive for the expansion of tau seeds into larger aggregates.

How can those seemingly contradictory pieces of evidence concerning the sequence of events on Aβ and tau dysfunction be reconciled into a single theory? It is possible that the disease initiates with soluble phase Aβ toxic species which trigger the cascade leading to tau pathology and overt neurodegeneration [99]. Sedimentation of soluble Aβ into plaques could just be a secondary phenomenon occurring later during disease progression, with no bearing of the number and location of the plaques on disease progression and on the severity of clinical features [87, 88, 91, 100].

Insights gained from genetics and neuropathology studies

Recent genetic findings on PD and AD and their relation with the transfer of tau and α-synuclein between cells

Several genome wide association studies (GWAS) conducted over the past five years [101, 102] [103, 104] have shown that a wide variety of processes contribute to the pathogenesis of neurodegenerative diseases such as AD and PD.

Within the past ten years, over 40 risk loci have been discovered for PD through GWAS [101, 102, 105, 106]. Even though at many of the associated loci the true functional gene is uncertain, many of the putative causative genes have functions related to the mitochondria, lysosomes and endosomes [107] and it is plausible that a primary dysfunction of those systems could induce the transfer of α-synuclein between cells. The cellular overload with misfolded protein that cannot be cleared efficiently would have to be disposed of through alternative methods, with a side effect being the spread (spillover) between cells. Such a process (MAPS) has been recently described for α-synuclein [74].

On the other hand, the situation appears to be less clear for AD. Genetics studies have pointed to a contribution of genes involved in inflammation pathways, lipid and cholesterol metabolism and vesicle recycling such as *ApoE, TREM2, CR1, ABCA7, CLU, CD33,* and *SORL1* [103, 108-110]. It is unknown how a dysfunction of such pathways could be associated with the transfer of tau and Aβ. One could hypothesize that lipid dysfunctions, or immunological deficiencies, may damage the cell membrane of neurons. This, in turn, could lead to the release of misfolded tau that is taken up by neighboring neurons, ultimately leading to spread of the pathology (Figure 3). In fact, two recent publications have shown that Aβ accumulates in response to infections by herpes simplex virus, Candida albicans and Salmonella enterica in an attempt to sequester the pathogenic agent [111-113]. However, this

process can go awry resulting in overt Aβ pathology triggering the cascade leading to AD.

In conclusion, the results from the genetic studies support that a wider system dysfunction causes PD and AD. It is important to determine through future studies how does the transfer and aggregation of misfolded protein fit into the larger picture.

A substantial proportion of cases deviate from the Braak pattern

There are several weaknesses regarding the Braak theories for the progression of neurodegenerative diseases [33, 114]. First, the sequential pattern for the development of LB pathology is not followed in a significant proportion of PD cases [115, 116] but also in dementia with Lewy bodies (DLB) [117]. Second, α-synuclein pathology is absent from some genetic forms of PD such as *parkin* and several cases of *LRRK2* [107]. Third, LBs can be found in healthy individuals [118]. Fourth, LB pathology does not correlate well with clinical features [119], similarly to Aβ pathology [91].

Deviations from the Braak hypothesis are also seen in the case of AD. According to mouse models used to study tau propagation that express human tau exclusively in the entorhinal cortex (EC) [24, 120, 121], the site to which tau spreads after the initial appearance of pathology in the EC is the dentate gyrus [122]. However, this pattern is not followed in humans because the dentate gyrus is affected at a later stage. Second, sometimes patients with AD do not fit in with the typical Braak staging as the hippocampus develops only mild tau pathology; alternatively, tau pathology can be present almost exclusively in the temporal lobe from where it eventually spreads to the hippocampus but nowhere else [123].

While neuropathology studies are directly informative only about deposition of misfolded proteins and not necessarily about cell-to-cell transmission, the aforementioned observations would support the notion that transfer of misfolded proteins from cell to cell are not responsible for the progression of the pathology seen in patients with AD and PD. However, it might just be the case that the PD and AD entities require reclassification to account for the different disease forms and related underlying pathogenetic mechanisms [107]; it is possible that α-synuclein and tau spreading is important in some forms of disease but has no or a minor role in others.

Concluding remarks

Since the first description of propagation phenomena in neurodegenerative diseases 10 years ago, it has become clear that this mechanism probably is not the main phenomenon involved in disease progression, and that the situation is more complex with crucial involvement of other mechanisms such as immunological dysfunction. We propose that protein propagation should be investigated in the context of a multimodal dysfunction within the brain rather as an isolated phenomenon (see outstanding questions).

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Figures

Figure 1: The definition of a "strain": Prion strains are prion protein species that can induce a certain type of pathology in the host organism, can be recovered and can induce the same type of pathology when introduced de novo in the naïve host organism. The passaging is still effective over several generations.

Figure 2: Assays used to study the propagation of prions and prionoids. Tau: A.The GFP-2a-tau genetic reporter is inserted into an AAV and can be used in vivo and in vitro to transfect primary neurons. B.HEK tau bionsensor FRET cells. HEK cells stably overexpress tau-CFP and tau-YFP. After adding tau fibrils or other tau preparations that can induce seeding, the cells develop inclusions that are FRET-positive. **α–synuclein:** D. HEK cells stably overexpressing α–synuclein. Addition of α–synuclein fibrils induces aggregation and phosphorylation of endogenous α–synuclein that can be detected using an anti-phospho-synuclein antibody. **α–synuclein and tau:** C. Injection of α–synuclein or tau fibrils in the brain of transgenic mice overexpressing α–synuclein or tau respectively induces widespread pathology that progresses through the brain. **Prions:** E. pK digestion assay: Brain is homogenized, treated with pK digestion and run on the western blot to compare the cleavage pattern with non-infections PrP. F. Protein misfolding cyclic amplification assay (PMCA): Misfolded protein is incubated with normal protein which undergoes conformational changes. Several treatment cycles with ultrasound break up the elongating misfolded structures thus leading to the production of more aggregating proteins. G. Scrapie cell assay: Misfolded PrP is added to N2a cell cultures that are passaged several times. The percentage of positive cells is determined through automatic cell counting. H. Mouse bioassay: Transgenic mice that overexpress PrP are injected with infectious PrPsc that induces widespread pathology.

Figure 3: Proposed model for the pathogenesis of AD: In this model, the core event in the pathogenesis of AD is the membrane damage in the dendrites of

neurons located within the entorhinal cortex. The following steps lead to and follow this central event: 1) Various events trigger the generation of toxic species of soluble Aβ. Such events include mutations within *APP*, *PS1* and *PS2* or infections. The location where this phenomenon occurs is unknown though it is most likely within the cortex. 2) The toxic Aβ species travel through the CSF throughout the brain but affect primarily the neurons in the entorhinal cortex that are selectively vulnerable. 3) The toxic Aβ species induce membrane damage on the dendrites which leads to the release of misfolded tau into the interstitial fluid and to the activation of a localized immune response and repair mechanism including molecules such as ApoE, TREM2, ABCA1, CR1 etc. The membrane damage is aggravated through a positive feedback loop. 4) The misfolded tau that has been released from the damaged neurons can be uptaken by synaptically connected, healthy neurons, leading to the propagation of the NFT pathology across neuronal networks, following the Braak staging pattern. 5) Soluble Aβ deposits throughout the brain in the form of plaques, which have little importance for the pathogenesis of the disease. The cortex is the location where the initial event leading to the generation of toxic Aβ species occurs; therefore, their concentration first reaches critical levels that allows their sedimentation as plaques within the cortex.